Molecular correlates between pituitary hormones and behavior

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MOLECULAR CORRELATES BETWEEN PITUITARY HORMONES AND BEHAVIOR

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I. INTRODUCTION

Hormones secreted by the pituitary play an important role in the behavioral adaptation of an organism to its environment. These hormones regulate homeostasis and create the conditions in which the animal can cope optimally with situational demands. Though much research has been directed at elucidating their mechanism of action in peripheral tissues, relatively little attention has been paid to the brain as a target for these hormones. However, it has been shown that pituitary principles are involved in a number of brain functions and that they are important for the maintenance of normal behavioral patterns. It was observed that learning is impaired after removal of the pituitary and that substitution of ad-

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renocorticotropic hormone (ACTH), α-melanocyte-stimulating hormone (α-MSH), or vasopressin restores this behavior (De Wied, 1969). Fragments of these hormones that lack the classic endocrine effects, were able to restore the impaired behavior. Similarly, peptides related to ACTH, MSH, or β-lipotropic hormone (β-LPH) were shown to influence behavior in intact animals (De Wied, 1969; De Wied *et al.*, 1978a). These peptides have not only been found in the pituitary but in many brain structures as well (Rossier *et al.*, 1977; Krieger *et al.*, 1977; Orwall *et al.*, 1979; Watson and Akil, 1980b). It was hypothesized that the pituitary releases peptides that are involved in the formation and maintenance of new behavior patterns. These so-called neuropeptides act directly on the central nervous system (CNS) (De Wied, 1969). In this view, environmental stimuli act to stimulate the release of the neuropeptides from the pituitary or from central cells. They modulate the activity of neuronal systems in the brain, and this altered activity finally results in behavioral adaptation of the animal to its environment (see Wiegant and De Wied, 1980).

This chapter reviews molecular correlates of the pituitary hormones. Relatively little is known about the molecular events that underlie the behavioral effects of the neuropeptides, though some biochemical data have been obtained on the action of peptide hormones in peripheral tissues. Therefore, some attention will be paid to this subject. The wealth of information that has been gathered on behavioral effects of pituitary hormones has forced us to limit ourselves to those behavioral effects that could be related to the available neurochemical data. We have, therefore, focused our attention on ACTH, MSH, β-LPH, and their fragments. The effects on pole-jumping avoidance behavior and grooming behavior are described as well as the opiate-like effects of these hormones.

For a more thorough review of behavioral aspects that are not covered in this chapter, the reader is referred to other papers (grooming behavior: Gispen and Isaacson, 1980; pituitary peptides and behavior: Wiegant and De Wied, 1980, De Wied and Gispen, 1977; lipotropin and CNS: Gispen *et al.*, 1977).

II. BEHAVIORAL EFFECTS OF PITUITARY PEPTIDE HORMONES

A. Acquisition and Retention of New Behavior Patterns

The implication that ACTH has CNS effects was first suggested by observations made on hypophysectomized rats. These animals showed impaired acquisition of shuttlebox avoidance behavior (De Wied, 1964; Applezweig and Baudry, 1955; Applezweig and Moeller, 1959). This behavioral impairment could not only be corrected by treatment with ACTH and α-MSH, but also by fragments of these hormones that are devoid of classical endocrine activity (De Wied, 1969; Bohus *et al.*, 1973). Furthermore, dexamethasone fails to restore shuttlebox

avoidance learning in hypophysectomized rats (De Wied, 1971), and avoidance behavior was not impaired in adrenalectomized rats. It was concluded that the behavioral effects are a consequence of a direct action of these peptides on specific areas of the CNS (De Wied, 1977; Greven and De Wied, 1973).

In intact rats, ACTH and congeners have been found to influence the acquisition and retention of new behavior patterns (see Wiegant and De Wied, 1980; De Wied, 1977). These peptides facilitate passive avoidance behavior (Levine and Jones, 1965; Lissák and Bohus, 1972; De Wied, 1974; Kastin *et al.*, 1973; Flood *et al.*, 1976) and delay the extinction of shuttlebox avoidance behavior (Greven and De Wied, 1973), pole-jumping avoidance behavior (De Wied, 1966), food-motivated behavior (Garrud *et al.*, 1974; Guth *et al.*, 1971), conditioned taste aversion (Rigter and Popping, 1976), and sexually motivated approach behavior (Bohus *et al.*, 1975). On the basis of these results it has been suggested that ACTH and related peptides are involved in motivational processes (De Wied, 1977).

These neuropeptides also affect learning and memory; they alleviate the amnesia that was induced by several treatments [inhalation of CO_2, administration of electroconvulsive shock, and treatment with protein synthesis inhibitors (Flexner and Flexner, 1971; Keyes, 1974; Rigter and Van Riezen, 1975; Rigter *et al.*, 1974)]. It has been suggested that the peptide affects memory storage (Gold and Van Buskirk, 1976; Flood *et al.*, 1976) or retrieval (Rigter *et al.* 1974), but motivational effects can not be excluded from most of the experimental paradigms used. So it was hypothesized (De Wied, 1977) that ACTH temporarily increases the motivational value of environmental stimuli, probably by selectively increasing a state of arousal in midbrain limbic structures. The possibility that stimulus-specific behavioral responses occur is thereby increased.

Although under certain conditions ACTH has been shown to improve acquisition of shock-motivated active avoidance behavior in intact rats (Beatty *et al.*, 1970; Guth *et al.*, 1971; Isaacson *et al.*, 1975), extinction of conditioned avoidance behavior seems to be more sensitive to the behavioral effects of the peptides (De Wied and Gispen, 1977). Results obtained on pole-jumping active avoidance behavior show the best dose-response relationship, therefore, structure–activity studies were performed on this paradigm to determine the essential elements required for the behavioral effect of ACTH.

It has been shown that $ACTH_{4-7}$ is the shortest active fragment with essentially the same behavioral potency as ACTH (Greven and De Wied, 1973; De Wied *et al.*, 1975). However, more activity sites are present in ACTH as the fragments $ACTH_{7-10}$ and $ACTH_{11-24}$ also contain some activity (Greven and De Wied, 1977). The residual potency observed for the sequence $ACTH_{7-10}$ could be increased to the same level as that of the reference peptide $ACTH_{4-10}$ by extending the C-terminal sequence to $ACTH_{7-16}$. Thus, the essential elements for avoidance behavior are not exclusively located in the region $ACTH_{4-7}$, but also occur in

other areas of the molecule (Greven and De Wied, 1977). Though both MSH and β-LPH contain the sequence $ACTH_{4-10}$ it was concluded that the structural requirements for the effects on pole-jumping avoidance behavior are more related to ACTH than to MSH or β-LPH. First, because of the second affinity site that becomes expressed after chain elongation to $ACTH_{7-16}$. Second, because of the potency of modified ACTH fragments (De Wied *et al.*, 1975; Greven and De Wied, 1977); the analog [Met(O)^4,D-Lys^8,Phe^9]$ACTH_{4-9}$ (Org 2766) is behaviorally 1000 times more active than $ACTH_{4-10}$, but possess 1000 times less MSH activity, and contains no opiate-like activity. Third, omission of either the glycyl residue in position 10 or the lysyl residue in position 16 is accompanied by a drastic decrease in potency (Greven and De Wied, 1977). For behavioral potency a doublet of basic lysine residues apparently is needed at exactly the same distance from the region $ACTH_{7-9}$ as in natural ACTH. This indicates that the structural requirements for behavioral activity in the pole-jumping test are more related to ACTH than to MSH or β-LPH. Extinction of pole-jumping avoidance was also used to assay the behavioral effect of C-terminal fragments of β-LPH (the endorphins). After subcuteneous (sc) injection, α-endorphin (αE; β-LPH_{61-76}) appeared to be the most potent peptide in delaying extinction of pole-jumping avoidance behavior (De Wied *et al.*, 1978a). On a molar basis it was 30 times as active as $ACTH_{4-10}$. After intraventricular administration, however, both peptides were equipotent, indicating that the difference in potencies after systemic administration is related to brain uptake mechanisms rather than to intrinsic behavioral effect.

The relatively weak activity of β-endorphin (βE; β-LPH_{61-91}) is probably the result of metabolic breakdown to fragments with opposite behavioral activity; γ-endorphin (γE; β-LPH_{61-77}) differs from αE by only one extra C-terminal amino acid, but it facilitates rather than delays the extinction of pole-jumping avoidance behavior (De Wied *et al.*, 1978b). Similarly, (des-tyrosine^1)-γ-endorphin (dTγE, β-LPH_{62-77}) was even more potent than γE in affecting avoidance behavior. A number of observations suggest that the influence of endorphins and of ACTH-like peptides on avoidance behavior takes place independently of opiate receptor sites in the brain. First, neither βE- nor ACTH-effects on pole-jumping avoidance behavior could be blocked by specific opiate antagonists (De Wied *et al.*, 1978a). Second, structurally modified peptides—e.g., [Met(O)^4,D-Lys^8,Phe^9] $ACTH_{4-9}$; Org 2766—have increased potency in the avoidance paradigm and have no opiate-like activity (Terenius *et al.*, 1975). Third, removal of the N-terminal tyrosine from the endorphins caused complete loss of opiate-like activity (on the guinea pig ileum) and destroyed the affinity for opiate binding sites (Guillemin *et al.*, 1976; Frederickson, 1977; De Wied *et al.*, 1978b), whereas the activity on pole-jumping active avoidance behavior was preserved (De Wied *et al.*, 1978a).

There is a striking similarity in effect on conditioned avoidance behavior

between dTγE and neuroleptic drugs such as haloperidol. The psychopharmacological actions of dTγE such as facilitation of extinction of pole-jumping avoidance behavior, attenuation of passive avoidance behavior (De Wied *et al.*, 1978b), interference with ACTH-induced grooming (Gispen *et al.*, 1980), and its activity in various grip tests (De Wied *et al.*, 1978b) are characteristic of neuroleptic drugs (Kováls and De Wied, 1978). In contrast, αE and related peptides have characteristics that resemble those of psychostimulants like amphetamine (Kovács and De Wied, 1978).

B. Opiate-Like Activity

The effects of C-terminal β-LPH fragments on extinction and grooming behavior (Section II,A and C) are obtained with amounts much lower than those needed to induce analgesia. Effects on extinction of pole-jumping avoidance behavior were obtained when amounts less than 1 μg were systemically injected, whereas after intracerebralventricular (icv) administration nanogram quantities are sufficient (De Wied *et al.*, 1978).

Profound analgesia was found after icv injection of higher quantities of βE (Bradbury *et al.*, 1976a), and icv administration of microgram doses of this substance in rats produced a naloxone-reversible catatonia (Bloom *et al.*, 1976). In its antinociceptive effects, βE appeared to be many times more potent than morphine. Similar effects of icv administrered Met-enkephalin (β-$LPH_{61\text{-}65}$) have been reported (Belluzzi *et al.*, 1976), but this peptide appears to be less potent than βE. This is probably due to enzymatic degradation *in vivo* (Hambrook *et al.*, 1976). The development of tolerance to βE was similar to that reported for morphine, and cross-tolerance between morphine, βE, or Met-enkephalin (Van Ree and De Wied, 1976; Bläsig and Herz, 1976) could be demonstrated. Morphine and βE also share similar dependence properties as assessed by naloxone-induced withdrawal signs (Wei and Loh, 1976; Loh *et al.*, 1976).

Not only peptides related to β-LPH have opioid activities. Neurophysiological evidence indicated that ACTH and β-MSH counteract the morphine-induced depression of spinal reflex activities *in vivo* (Zimmerman and Krivoy, 1973). $ACTH_{1\text{-}24}$ also counteracts morphine effects *in vitro* (Zimmermann and Krivoy, 1974) indicating a morphine–peptide interaction at the level of CNS. Furthermore, purified ACTH and $ACTH_{1\text{-}24}$ antagonized the analgesic effect of moprhine (Paroli, 1967; Gispen *et al.*, 1976a). The peptides $ACTH_{1\text{-}24}$, $ACTH_{1\text{-}16}$, $ACTH_{5\text{-}16}$, $ACTH_{5\text{-}14}$, and [D-Phe^7]$ACTH_{4\text{-}10}$ reduced the analgesic effect of morphine, as measured by the hot-plate test, by 50% (Gispen *et al.*, 1976a). $ACTH_{4\text{-}10}$ was less active, whereas $ACTH_{11\text{-}16}$, $ACTH_{11\text{-}17}$, and $ACTH_{11\text{-}24}$ were inactive. These results suggest that the sequence $ACTH_{4\text{-}10}$ may contain the active site; a secondary site may provide additional affinity without exerting intrinsic activity.

C. Excessive Grooming Behavior

It has long been known that birds and small mammals display enhanced grooming behavior in situations in which novel or conflicting environmental stimuli are present (Sevenster, 1961; Tinbergen, 1940; Bolles, 1960). Of course, these same stimuli are known to activate the pituitary–adrenal system (Mason, 1968) implying that this system is involved in grooming induction. However, since hypophysectomized rats still show novelty-induced grooming (Jolles *et al.*, 1979a), the pituitary gland can not be directly involved. Two lines of evidence implicate centrally active ACTH as playing a role in the induction of grooming. First, icv administration of antibodies to ACTH reduced novelty-induced grooming (Dunn *et al.*, 1979). Second, intraventricular injection of ACTH or its N-terminal fragments produced an enhanced display of grooming (Ferrari *et al.*, 1963; Izumi *et al.*, 1973; Gispen *et al.*, 1975; Wiegant and Gispen, 1977). In view of the short latency of icv-administered ACTH and since its effects are independent of its endocrine activity (Gispen *et al.*, 1975), this supports a direct CNS effect for ACTH in inducing excessive grooming. Furthermore, systemic administration of the peptide fails to induce grooming, once again implying a direct CNS effect. It has been suggested that grooming dearouses the organism after its activation by ACTH (Jolles *et al.*, 1979a,b; Delius, 1970; Delius *et al.*, 1976).

Structure–activity studies have been performed to determine the elements necessary for ACTH-induced grooming. $ACTH_{1-16}$, α-MSH, and β-MSH were as potent as $ACTH_{1-24}$, but $ACTH_{1-13}NH_2$ was less active (Gispen *et al.*, 1975). As in the avoidance studies (Section II,A), the shortest sequence possessing grooming-inducing activity was $ACTH_{4-7}$ (Wiegant and Gispen, 1977). $ACTH_{4-10}$ and $ACTH_{1-10}$, peptides containing full information for the effect on avoidance behavior, were inactive in the excessive grooming test, but D-Phe^7 substitution rendered them active (Gispen *et al.*, 1975). As the fragments $ACTH_{1-13}NH_2$, $ACTH_{5-14}$, and $ACTH_{5-16}$ also showed grooming-inducing activity, a secondary site may be present beyond the tenth amino acid. This site lacks intrinsic grooming-inducing activity as may be concluded from the inactivity of the fragments $ACTH_{7-16}$ and $ACTH_{11-24}$. In contrast, these fragments are active in the avoidance paradigm (Section II,A), so these data suggest that the peptide has different mechanisms of action in the two behavioral models. In fact, the grooming response may relate more to C-terminal β-LPH than to ACTH. For instance, icv-injected βE is more potent than $ACTH_{1-24}$ in producing excessive grooming (Gispen *et al.*, 1976b); the opioid was able to induce the grooming response in doses as low as 10 ng. The nature of the excessive grooming induced by β-endorphin is somewhat different from the ACTH-induced behavior in that the β-endorphin grooming is frequently interrupted by signs of excitation (quick movements of body and head, jumping, gnawing, and body shakes). Shortening

the βE from the C-terminal end resulted in a rapid and progressive loss of activity. Both αE and γE possessed slight grooming-inducing potency, while $\beta LPH_{61\text{-}69}$ was the shortest active sequence. Met-enkephalin was inactive, even if injected in high doses (Gispen *et al.*, 1976b). The des-Tyr fragments, that lack opiate-like activity (Guillemin *et al.*, 1976; Frederickson, 1977; De Wied *et al.*, 1978b) were also inactive, again underscoring the "opiate-like" character of peptide-induced grooming. Similarly, peripheral administration of specific opiate antagonists (naloxone, naltrexone) completely inhibited the grooming induced by $ACTH_{1\text{-}24}$ (Gispen and Wiegant, 1976) and by βE (Gispen *et al.*, 1976b). Recently it was found that an "acute tolerance" develops after $ACTH_{1\text{-}24}$ (Jolles *et al.*, 1978) and βE administration (Wiegant *et al.*, 1978). After icv injection these peptides were not able to induce grooming when given 1–8 h after a previous injection of the peptide. A cross-tolerance was demonstrated for ACTH and βE, and also for ACTH and morphine, and systemic treatment with naloxone at the time of the first icv injection prevented the development of the single-dose tolerance (Jolles *et al.*, 1978). These results suggest that a close relationship may exist between ACTH- and endorphin-sensitive structures in the CNS.

D. Discussion

ACTH, MSH, β-$LPH_{61\text{-}91}$, and their fragments affect avoidance behavior, analgesia, and grooming behavior as described in the previous sections. It was found that the structural requirements for the effects on pole-jumping avoidance behavior were less exacting than those needed for grooming and opiate-like activities (Fig. 1). The fragment $ACTH_{4\text{-}10}$, which is present in ACTH, MSH, and β-LPH, contains the prime affinity site; $ACTH_{4\text{-}7}$ is the shortest sequence possessing activity in the avoidance paradigm and in groooing behavior. $ACTH_{4\text{-}10}$ was fully active on pole-jumping avoidance behavior, but was inactive in grooming test and analgesia. D-Phe^7 substitution rendered this peptide active in the latter test but reversed its effect on pole-jumping avoidance behavior. A second affinity site was found C-terminal to the sequence $ACTH_{4\text{-}7}$. This second site exhibited activity in pole-jumping avoidance behavior, but not in grooming and analgesia; $ACTH_{7\text{-}16}$ and $ACTH_{11\text{-}24}$ were active on avoidance behavior, but not on grooming and analgesia. It was concluded that the effects on avoidance behavior relate more to ACTH than to MSH or β-LPH; in contrast, effects on grooming may relate more to the opiate-like activity and to C-terminal β-LPH.

The metabolic stability of the peptides certainly contributes to the behavioral potency (Greven and De Wied, 1977; Witter *et al.*, 1975). Elongation of the peptide chain and modification of its structure by amino acid substitution can increase the *in vitro* half-life. However, the enhanced effect of [D-Phe^7]$ACTH_{4\text{-}10}$ and [D-Phe^7]$ACTH_{1\text{-}10}$ on grooming behavior can not be explained by an im-

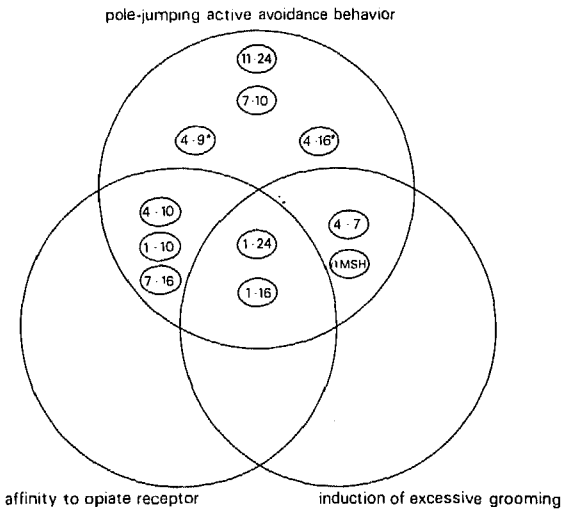

Fig. 1. Venn diagram showing the distribution of a number of ACTH fragments over three overlapping circles. Each circle encloses a collection of ACTH fragments, active on a particular test of CNS activity. This figure suggests that different parts of the ACTH molecule can interact with receptors mediating different functions. The data on "affinity for opiate receptor" are described in Section III,E. The fragments [MET(O)^4, D-Lys^8, Phe^9] $ACTH_{4-9}$, and [Met(O)^4, D-Lys^8, Phe^9, D-Lys^{11}] $ACTH_{4-16}$ are denoted as (4-9*) and (4-16*), respectively.

proved metabolic stability as [D-Arg^8]$ACTH_{4-10}$ and [Met(O)^4, D-Lys^8, Phe^9]-$ACTH_{4-9}$ were inactive in the grooming test (Grispen *et al.*, 1975). So, the strong behavioral potentiation after structural modification may reflect increased activity and/or intrinsic affinity for the receptor sites in the CNS.

In conclusion, there is a redundancy in behavioral information in ACTH, MSH, and β-LPH. These, and possibly other peptides are derived from the same large precursor molecule (Mains *et al.*, 1977; Peng Loh, 1979). The demonstration of specific peptidases in brain membranes (Burbach *et al.*, 1979, 1980; Gráf *et al.*, 1976; Bradbury *et al.*, 1976b) suggest that these peptides in turn may serve as precursor for a series of shorter sequences with a variety of behavioral activities.

III. MOLECULAR CORRELATES OF PITUITARY PEPTIDE HORMONES

From behavioral studies it was concluded that ACTH and related peptides act directly on CNS structures. Indeed, ACTH, α-MSH, and the endorphins have been found in the pituitary as well as in many brain structures (Rossier *et al.*,

1977; Krieger *et al.*, 1977; Orwoll *et al.*, 1979; Watson and Akil, 1980b). A functional connection between ACTH and endorphin systems in the brain is suggested by observations that β-LPH, βE, and ACTH-like peptides can occur in the same neuronal cells (Watson and Akil, 1980a,b; Watson *et al.*, 1978a,b; Pelletier and Leclerc, 1979). Though ACTH-like immunoreactive material has been demonstrated throughout the brain (Krieger *et al.*, 1977), a function for ACTH as a classical neurotransmitter in the CNS lacks experimental support. Currently the influence of ACTH-like neuropeptides on brain neuronal activity is best formulated as neurohormonal or neuromodulatory (Barchas *et al.*, 1978; Gispen *et al.*, 1979). Since ACTH-like peptides have a direct effect on the CNS, many investigators have attempted to relate the behavioral effects of these peptides to a direct effect on neuronal cells. It is expected that the neuropeptide binds to a specific receptor on the cell membrane. Although high affinity binding sites for ACTH and related peptides have as yet not been demonstrated in the brain (Witter, 1980), the multiplicity and specificity of the ACTH–CNS interactions by themselves point to the presence of specific ACTH receptors in the brain. It is conceivable that ACTH interacts with neuronal membranes resulting in events that are similar to those found for other polypeptide hormones or neurotransmitters. The evidence for such a neurochemical action of the neuropeptides will be reviewed in the following sections.

A. Neurotransmitters

The early work of Weiss *et al.* (1970) and Hökfelt and Fuxe (1972) led investigators to assume that ACTH-like peptides would specifically alter the turnover of noradrenaline (NA) in the brain. The prediction was that delay of extinction of a conditioned avoidance response correlates with an increased NA turnover, whereas peptides having the opposite effect would lead to a decreased rate of NA turnover. More recent reports have not been able to substantiate this notion (Versteeg, 1973; Leonard, 1974; Iuvone *et al.*, 1978; Kostrzewa *et al.*, 1975). Similar effects of ACTH on brain serotonin metabolism are by no means convincing (Leonard, 1974; Leonard *et al.*, 1976; Spirtes *et al.*, 1975; Telegdy and Kovács 1979a,b), though Ramaekers *et al.* (1978) reported that the effects of $ACTH_{4\text{-}10}$ and [D-Phe^7]$ACTH_{4\text{-}10}$ on passive avoidance behavior correlate to changes in hippocampal serotonin levels. Brain dopamine (DA) has been related to ACTH-induced excessive grooming (Wiegant *et al.*, 1977b; Cools *et al.*, 1978) and acetylcholine (ACh) turnover in the hippocampus may relate to the stretching and yawning syndrome induced by $ACTH_{1\text{-}24}$ and α-MSH (Wood *et al.*, 1978, 1979).

A large number of reports have been published concerning the interaction of endorphins, enkephalins, and their analogues with the metabolism of classical neurotransmitters (for a recent review see Versteeg, 1980). Although a majority

of these reports indicate that these peptides increase striatal DA metabolism, there are also reports presenting conflicting evidence. One possible explanation of these conflicting reports stems from the work of Van Loon and Kim (1978). They found that the effects of βE on striatal DA are time-dependent resulting in an accelerated and then a decelerated utilization of DA. It has been concluded that part of these effects are mediated via interaction with presynaptic opioid receptors of the nigrostriatal DA terminals (Biggio *et al.*, 1978a,b). However, this accounts for only one-third of the opioid receptors of the striatum (Pollard *et al.*, 1977, 1978; Schwartz *et al.*, 1978, 1979; Carenzi *et al.*, 1978; Trabucchi *et al.*, 1979). It is likely that several mechanisms are involved in the interaction of these peptides with striatal DA activity.

The endorphins and enkephalins also influence brain ACh turnover via opioid receptors. βE modulates hippocampal ACh turnover. 10 μg per rat of βE administered icv decreased ACh turnover in the hippocampus, nucleus accumbens, globus pallidus, and cortex, but not in the caudate nucleus (Moroni *et al.*, 1977). In addition, Botticelli and Wurtman (1979) found that this same amount of βE also given icv elevated hippocampal ACh levels. In both studies opioid antagonists inhibited the effects.

Met-enkephalin also influences brain ACh release. Met-enkephalin reduced the K^+-stimulated transmitter release from hippocampal slices (Subramarian *et al.*, 1977) and reduces spontaneous release from cerebral cortex (Jhamandas *et al.*, 1977). However, in striatal slices, Met-enkephalin methylester and two other analogues increase ouabain-induced ACh release (Vizi *et al.*, 1977; Harsing *et al.*, 1978). These effects were all prevented by naloxone pretreatment, indicating the involvement of opioid receptors.

Although enkephalins also have effects on catecholamine metabolism in other brain regions, Versteeg (1980) concludes that our knowledge of these effects is not only fragmentary, but that the significance of the observed changes is obscure.

It is apparent from the previous discussion that ACTH, endorphins, and enkephalins all influence brain neurotransmitter metabolism. In many cases the results are confusing and apparently contradictory perhaps because different species, brain regions, methods as well as peptides (or analogues) were used in the different studies. However, it must also be considered that the effects of these peptides on neurotransmitter metabolism reflect indicrect consequences of peptide action. In the following sections we summarize the evidence that these peptides interact directly with CNS neurons and speculate on the possible mechanisms of action of these peptides.

B. Cyclic Nucleotide Metabolism

The mechanism of action of the neuropeptides is not yet understood. However, evidence from studies of neuropeptide action in peripheral tissues suggests that

these peptides may act via cAMP. In the second messenger model of Sutherland and Robinson (1966), interaction of the hormone (first messenger) with its membrane receptor activates adenylate cyclase giving rise to an increased intracellular level of cAMP. This cAMP acts as a second messenger by activating various protein kinases.

Cyclic nucleotides are thought to mediate the action of ACTH. Evidence has accumulated that cAMP is involved in the process of steroidogenesis in the adrenal cortex, and of lipolysis in the fat cell (Fain, 1973; Halkerston, 1975). For example, the steroid production that is induced by ACTH is accompanied by a rise in intracellular receptor-bound cAMP (Podesta *et al.*, 1979) and by activation of cAMP-dependent protein kinases (Kudlow *et al.*, 1980).

However, the sole involvement of cAMP in the process of steroidogenesis has been questioned. It has been found that the stimulation of cAMP production in adrenal cortex was dependent on the presence of Ca^{2+} (Bär and Hechter, 1969; Glossman and Gips, 1975, 1976). Bristow *et al.* (1980) found that ACTH may induce steroidogenesis via two different mechanisms: one involves the production of cAMP and the other acts via Ca^{2+} and cGMP. A similar finding was reported by Perchellet and Sharma (1979). They found no steroidogenesis in the absence of Ca^{2+}, even when high concentrations of the hormone were present. Exogenous cGMP could stimulate steroidogenesis.

There have been very few reports concerning the central effect of peptide hormones on brain cyclic nucleotides. It seems likely, however, that there is a parallel between the peripheral and the central mechanism of action. ACTH has been occasionally included in studies on the effect of putative neurotransmitters and hormones on brain adenylate cyclase. Burkard and Gey (1968) and Von Hungen and Roberts (1973) did not detect an effect of ACTH in cell-free membrane preparations. In addition, Forn and Krishna (1971) did not observe an effect of the peptide on cAMP accumulation in slices of cerebral cortex, cerebellum, and hypothalamus. Rudman and Isaacs (1975; Rudman, 1976) were the first to present indirect evidence that ACTH-like peptides might affect brain cyclic nucleotide levels *in vivo*. They showed that intracisternal injection of ACTH or β-MSH in rabbits increased the level of cAMP in the cerebrospinal fluid (CSF). In other studies it was found that chronic treatment of intact as well as hypophysectomized rats with β-MSH resulted in an increased level of cAMP (but not cGMP) in the occipital cortex (Christensen *et al.*, 1976; Spirtes *et al.*, 1978). Direct evidence for the involvement of cAMP in the action of ACTH on the brain was provided by Wiegant *et al.*, (1979). These authors found that the hormone stimulated the production of cAMP in striatal slices *in vitro*. In a broken cell preparation, however, a biphasic dose-response relationship was found. Low concentrations of the peptide (10^{-7}, 10^{-6} *M*) stimulated the cAMP accumulation, but high concentrations (10^{-5}, 10^{-4} *M*) inhibited the cAMP accumulation (Wiegant *et al.*, 1979). The effect of Ca^{2+} was similar to that observed in adrenal cortex: EGTA reduced the basal activity to 20% and abolished the effect of the

hormone (Fig. 2). The authors showed that the hormone effect is not due to interaction with DA receptors as the DA receptor blocker ergometrine did not counteract the ACTH-inhibited adenylate cyclase activity. Moreover, it was improbable that the observed peptide effect was due to an interaction with the opiate receptor, as the inhibitory effect of ACTH was not counteracted by the specific opiate antagonist naltrexone (Fig. 2; Wiegant *et al.*, 1979). Interestingly, structure activity studies revealed a correspondence between the effects on cAMP production and those on the induction of excessive grooming in rats *in vivo:* $ACTH_{1-16}NH_2$ and $ACTH_{4-7}$ were active, whereas $ACTH_{11-24}$, $ACTH_{1-10}$, $ACTH_{4-10}$, and the D isomers were not (Wiegant *et al.*, 1979). Also, icv injection of $ACTH_{1-16}NH_2$ *in vivo* significantly elevated the cAMP level in the septal area (Wiegant *et al.*, 1979). This area is thought to be an important target for behaviorally active ACTH-like peptides. Such peptides have been shown to specifically accumulate in that region, when administered intracerebroventricularly (Verhoef *et al.*, 1977). Taken together, these studies support the notion that ACTH, in addition to its effects on the production of cyclic nucleotides in peripheral tissue is able to influence their metabolism in the CNS.

C. Protein Phosphorylation

It has been suggested that changes in the state of phosphorylation of membrane proteins may govern the ion permeability of the neuronal membrane (Heald, 1962) and thus may play a key role in determining the functional activity of the neuron (Greengard, 1976, 1978). Alternatively, phosphorylation of membrane enzymes may alter their activity, thereby changing the metabolism of important membrane components (for review on enzyme phosphorylation, see Krebs and Beavo, 1979).

There have been very few reports concerning the effects of peptide hormones on brain protein phosphorylation; enkephalins (Davis and Ehrlich, 1979) and β-endorphin (Ehrlich *et al.*, 1980) have been found to inhibit the phosphorylation of synaptosomal membrane proteins. Also in intact hippocampal slices effects of enkephalins have been noted (Bär *et al.*, 1980). Effects of ACTH-like peptides on brain protein phosphorylation have been described by Zwiers *et al.* (1976). These authors showed that ACTH exerts a dose-dependent inhibitory effect on the phosphorylation of proteins in synaptosomal plasma membrane fractions from rat brain (Zwiers *et al.*, 1976, 1978). The phosphorylation of one of these proteins (B-50 protein) was especially sensitive to ACTH. Structure–activity studies with ACTH-like peptides on the endogenous phosphorylation of B-50 showed that $ACTH_{1-24}$ and $ACTH_{1-16}$ are equally active, and that $ACTH_{1-13}$ and $ACTH_{5-18}$ also possess activity. The sequences 1–10, 11–24, 4–10, 5–16, and 7–16 were inactive (Zwiers *et al.*, 1978). Clearly, this structure–activity relationship is very similar to that found for the induction of excessive grooming

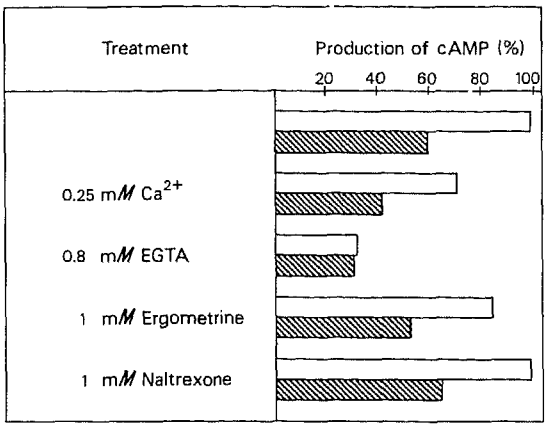

Fig. 2. Effects of various agents on the inhibition of adenylate cyclase in synaptosomal plasma membranes by 100 μM $ACTH_{1\text{-}24}$. These data show that calcium is required for the inhibitory effect of $ACTH_{1\text{-}24}$ and that this effect is not reversed by opiate (naltrexone) or dopamine (ergometrine) antagonists. The data upon which the table is based are from Wiegant *et al.* (1979). A synaptosomal plasma membrane fraction was incubated for 10 min in medium containing [^{3}H]ATP in the presence (shaded bars) or absence (open bars) of 100 μM $ACTH_{1\text{-}24}$. The agents to be tested were dissolved in incubation medium.

(Gispen *et al.*, 1979; Fig. 3). Also when ACTH was administered *in vivo* (icv) dose-dependent effects on the *in vitro* phosphorylation of B-50 were obtained (Zwiers *et al.*, 1977). ACTH specifically inhibited the B-50 kinase and did not affect the phosphoprotein phosphatase. Interestingly, the presence of Ca^{2+} was essential for B-50 kinase activity as no B-50 labeling was obtained after removal of endogenous Ca^{2+} with EGTA (Gispen *et al.*, 1979).

A more general inhibitory effect of ACTH was obtained in Triton-solubilized membrane fractions (Zwiers *et al.*, 1979) and in the presence of cytoplasmic proteins (Jolles *et al.*, 1980c). This suggests that the ACTH-sensitive protein kinase has a broad specificity and is able to phosphorylate other proteins when given access to them (Jolles *et al.*, 1981b).

The B-50 kinase and its protein substrate were isolated in soluble form from the membrane and purified by DEAE-celluose chromatography (Zwiers *et al.*, 1979) and ammonium sulfate precipitation (Zwiers *et al.*, 1980a). The highly purified enzyme complex was still sensitive to ACTH. The B-50 protein (MW 48 K; I.E.P. 4.5) and its kinase (MW 71 K; I.E.P. 5.5) were identified by isoelectric focusing (Zwiers *et al.*, 1980a). The B-50 protein was very susceptible to proteolytic breakdown (Zwiers *et al.*, 1980b). It yielded a large (MW 45 K) and a small (MW 1650) polypeptide. The small basic peptide has 15 amino acids. It was called Phosphorylation Inhibiting Peptide (PIP) due to its inhibitory effect on B-50 phosphorylation. Interestingly, icv injection of PIP into rats elicited a

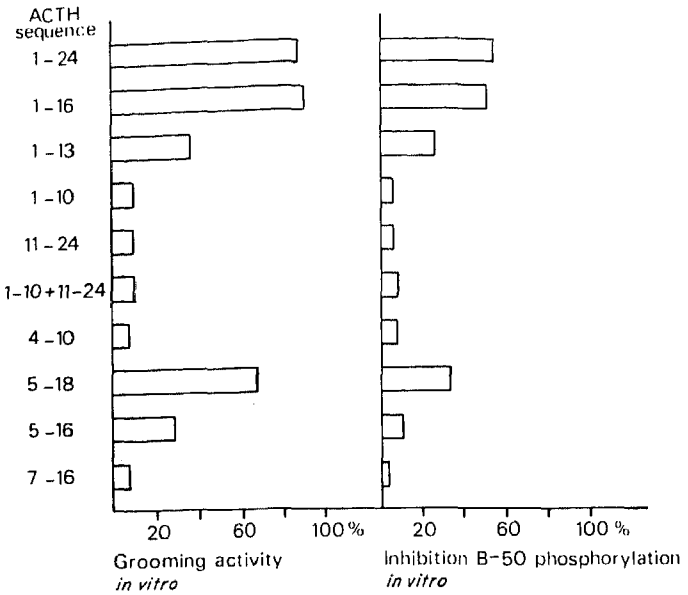

Fig. 3. The effect of $ACTH_{1-24}$ on excessive grooming *in vivo*, and inhibition of membrane protein phosphorylation *in vitro:* structure-activity relationship. This shows the correlation between the effects of ACTH fragments on these two phenomena. The data upon which the table is based are from Gispen *et al.* (1975, 1979) and Zwiers *et al.* (1978). The grooming was elicited by intraventricular administration of doses equimolar to 3 μg $ACTH_{1-24}$. Phosphorylation was studied in a synaptosomal plasma membrane fraction incubated in medium containing [γ-^{32}P]ATP for 20 sec and incorporation into proteins was determined. The results are expressed as percentage of control incubations.

grooming response that was similar to the ACTH-induced behavior (Zwiers *et al.*, 1980b). This again relates the *in vitro* effects of this peptide to its *in vivo* effects on grooming behavior.

Recent evidence suggests that the B-50 kinase/B-50 complex may have a function in the polyphosphoinositide metabolism in the membrane (Section III,D).

D. Polyphosphoinositide Metabolism

A special class of membrane phospholipids, the polyphosphoinositides, has been implicated in the metabolism of Ca^{2+} at the cell membrane (for reviews see Michell, 1975; Hawthrone and Pickard, 1979). Both the binding of Ca^{2+} to the membrane and its permeability are thought to be regulated by the metabolism of phosphatidylinositol (PI) and its phosphorylated derivates phosphatidylinositol 4-phosphate (DPI) and phosphatidylinositol 4,5-diphosphate (TPI; Fig. 4). The

relatively high content of DPI and TPI in brain tissue and their rapid metabolism (Hawthorne and Kai, 1970) suggest that these lipids may play an important role in brain cell membrane function. The breakdown of brain PI and its rapid resynthesis via phosphatidic acid (PA) has first been described by Hokin and Hokin (1959). This so-called PI response has been observed in various tissues after receptor activation by hormones and neurotransmitters that utilize Ca^{2+} as their intracellular second messenger (Michell, 1975). An enhanced production of DPI and TPI in adrenal cortex was found after administration of ACTH to rats *in vivo* (Farese *et al.*, 1979). In the authors view, this suggests a direct relation between poly(PI)metabolism and ACTH-induced steroidogenesis, in view of the stimulatory effect of polyphosphorylated lipids (DPI, TPI, cardiolipin) on adrenal pregnenolone synthesis *in vitro* (Farese and Sabir, 1979). The phosphoinositides also mediate the effects of neurohypophyseal hormones.

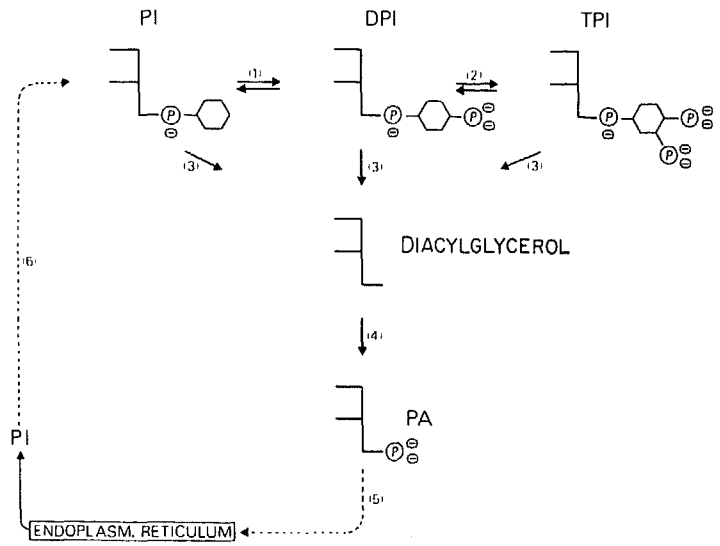

Fig. 4. Metabolism of polyphosphoinositides. Phosphatidyl-*myo*-inositol (PI) and its phosphorylated derivates phosphatidyl-*myo*-inositol 4-phosphate (DPI) and phosphatidyl-*myo*-inositol 4,5-diphosphate (TPI) are rapidly interconverted by lipid kinases and/or phosphomonoesterases (1 and 2). A poly(PI)-specific phosphodiesterase can hydrolyze the phosphodiesterlinkage in a phospholipase C-type manner, thereby yielding 1,2-diacylglycerol (1,2-daG) (3). This substance is rapidly phosphorylated to phosphatidic acid (PA) (4). All these reactions take place at the plasma membrane. Resynthesis of PI from PA takes place in the endoplasmic reticulum, and the transport of the lipid between plasma membrane and intracellular membrane is performed by phospholipid-exchanging proteins (5, 6). The interconversion of these compounds play an important role in calcium metabolism and neuronal cell function.

Effects of pituitary peptide hormones on polyPI metabolism in rat brain have recently been found. A preliminary study showed that both $ACTH_{1-24}$ and lysine-vasopressin (LVP) affected these lipids in a prelabeled synaptosomal fraction (Jolles *et al.*, 1979c). In subsequent studies a lysed synaptosomal fraction was used. It was found that $ACTH_{1-24}$ stimulated the formation of TPI, while at the same time inhibiting the production of PA (Jolles, 1980). These effects were evident after very short time periods (5 sec after the start of the incubation). The peptide was found to act on the lipid kinases, but not on the respective phosphatases (Jolles *et al.*, 1981b). Lipid kinase activity showed a strong calcium dependency (Jolles, 1980): activity was maximal at zero calcium, decreased rapidly as the calcium concentration increased, and was completely inhibited at Ca^{2+} concentrations greater than 1 m*M*. Similarly, the ACTH stimulation of TPI formation was also very sensitive to Ca^{2+}. The ACTH effect was maximal in the absence of Ca^{2+} resulting in a 3.5-fold stimulation of TPI formation. At 0.1 m*M* Ca^{2+}, the ACTH effect was reduced such that it gave only a twofold stimulation of TPI labeling. At 1 m*M* Ca^{2+} the effect of ACTH was completely abolished.

The evidence described so far suggested that a relation might exist between protein phosphorylation and poly(PI) metabolism. Direct evidence in support of this notion was obtained; the ACTH-sensitive B-50 kinase/B-50 protein complex (Section III,C) was solubilized from synaptosomal plasma membranes and purified by DEAE-cellulose chromatography. It appeared that the B-50 kinase/B-50 peak cochromatographed with a peak of DPI kinase activity (Jolles, 1980; Jolles *et al.*, 1980). Moreover, these peak fractions were also able to use DPI as a substrate for the production of PA. This indicated a combined phosphodiesterase/1,2-diacyclglycerol (1,2-daG) kinase activity. Addition of $ACTH_{1-24}$ to the DEAE-cellulose peak fractions inhibited both the phosphorylation of B-50 and the formation of PA, and stimulated the formation of TPI (Jolles *et al.*, 1980). The same peptide effects had been found in intact membranes (Jolles, 1980). Further fractionation of the DEAE peak fractions by ammonium sulfate precipitation isolated the PDE/1,2-daG kinase activity in the ASP 0–55% fraction (Jolles, 1980), but some of the DPI kinase activity was still present in the ASP 55–80% fraction (Jolles, 1980). As had been shown by Zwiers *et al.* (1980a), this fraction is enriched with respect to B-50/B-50 kinase. An inverse relationship was found between B-50 phosphorylation and TPI formation in this purified enzyme fraction; when the B-50 phosphorylation was increased (by prephosphorylation) TPI formation was decreased, but when B-50 phosphorylation was inhibited (by $ACTH_{1-24}$), TPI formation was stimulated (Jolles *et al.*, 1980). We suggested that the B-50 kinase and the DPI kinase originate from one enzyme complex of which B-50 is a regulatory unit. Autophosphorylation of this subunit would regulate the lipid-phosphorylating activity of the enzyme complex (Jolles, 1980; Jolles *et al.*, 1980, 1981b).

In order to further elucidate the mechanism of the effects of ACTH on neuronal membrane lipid phosphorylation, structure activity studies have been performed with ACTH fragments in a lysed synaptosomal fraction (Jolles *et al*, 1981a). A direct correlation was found between the effects of ACTH fragments on PA and TPI phosphorylation and those obtained on B-50 phosphorylation in synaptosomal plasma membranes, again stressing the relationship between B-50 phosphorylation and polyPI metabolism. The effects on TPI formation (stimulation) and PA formation (inhibition) decreased in the order $ACTH_{1-24} > ACTH_{5-18} > ACTH_{1-16} > ACTH_{1-13}$. $ACTH_{1-10}$ was ineffective (Jolles *et al.*, 1981a). Both the sequences $ATCH_{4-7}$ and $ACTH_{11-16}$ may contain information that is critical for the nature of the *in vitro* effects. Loss of the doublet of lysine residues at the position 15 and 16 abolished the effect on polyPI metabolism (Jolles *et al.*, 1981a). Similarly, βE inhibited the formation of PA, whereas γE (β-LPH_{61-77}), αE, the des-Tyr^{61}-derivates, and the enkephalins were not active. It was concluded (Jolles *et al.*, 1981a) that the structure-activity relationship correlates with the *in vivo* activity of the peptides on grooming behavior (Gispen *et al.*, 1975, 1976b), pole-jumping avoidance behavior (Greven and De Wied, 1973, 1977; De Wied *et al.*, 1975), and counteraction of morphine-induced analgesia (Gispen *et al.*, 1976a). We suggested that the correspondence in effects of ACTH and β-endorphin *in vivo* (grooming behavior and opiate-like activity on TPI and PA) might result from the structural similarity of $ACTH_{5-16}$ and β-LPH_{78-91}. Interestingly, the opiate antagonist naloxone had an intrinsic effect on the metabolism of TPI and PA (an inhibition, Jolles 1980) but failed to antagonize the effects of $ACTH_{1-24}$ on TPI and PA formation. It seems that the effects on avoidance behavior *in vivo* correlate with those on DPI metabolism *in vitro* (Section IV) though more research should be directed at elucidating this question (Jolles *et al.*, 1981c).

E. Brain Opiate Receptors

$ACTH_{1-28}$ and $ACTH_{4-10}$ have appreciable affinity for stereospecific opiate binding sites in brain synaptosomal plasma membranes as studied by competition with dihydromorphine (Terenius, 1975). From structure-activity studies it was concluded that $ACTH_{4-10}$ contains the active site. A second affinity site might be present C-terminal to the sequence 4-10 (Gispen *et al.*, 1976a, Terenius *et al.*, 1975). Vasopressin and α-MSH showed no affinity for the opiate receptor (Terenius, 1975; Terenius *et al.*, 1975). Analysis of the binding characteristics of $ACTH_{1-24}$ revealed a relatively low selectivity for agonist and antagonist binding sites, comparable to a partial agonist-antagonist like nalorfine (Terenius, 1976). The magnitude of the affinity constants of ACTH fragments (IC-50) is in the order of 10^{-5}–10^{-6} *M*, indicating that these peptides may not be powerful endogenous ligands for opiate receptors. Strikingly, the *in vitro* binding charac-

teristics of βE suggest that this peptide also has mixed agonist–antagonist character (Bradbury *et al.*, 1976c). β-Endorphin is also very potent in inducing excessive grooming *in vitro*. In contrast, Met-enkephalin has pronounced agonist properties *in vitro* (Terenius, 1976; Bradbury *et al.*, 1976c) and has no grooming-inducing activity (Gispen *et al.*, 1976b).

These data and the recent findings that ACTH-like peptides have affinity for βE binding sites *in vitro* (Akil *et al.*, 1980) support the notion that ACTH-like peptides interfere with opiods at the level of the CNS (Wiegant *et al.*, 1977a). Similar conclusions have been reached by others. It has recently been proposed (Jacquet, 1978) that the effects of morphine are mediated by two classes of receptor. One, which is stereospecific and naloxone-sensitive (endogenous ligand: β-endorphin) and the other which is nonstereospecific and naloxone-insensitive. ACTH may be the endogenous ligand for the second receptor.

F. Interaction with Membrane Lipids

Membrane lipids may be important for opiate receptor binding. Anionic lipids like cerebroside sulfate are claimed to be part of the opiate receptor (Loh *et al.*, 1978). β-Endorphin specifically interacts with negative lipids (Wu *et al.*, 1979); this peptide formed an α-helix in solutions of cerebroside sulfate, PA, and PS. Ca^{2+} counteracted the helix-forming tendency and the authors suggested that micelles of the amphiphilic lipid cluster around the peptide chain, thus stabilizing the conformation by hydrophobic and hydrophilic interactions (Wu *et al.*, 1979). Likewise, it has been argued that $ACTH_{4-10}$, which is all probability is present in water as a random coil, may assume an α-helix structure in the more hydrophobic environment of the receptor site (Greven and de Wied, 1977). Also phosphatidylserine (Hoss *et al.*, 1977; Abood *et al.*, 1978) and long-chain-polyunsaturated fatty acids (Abood *et al.*, 1978) are claimed to modulate opiate receptor binding, opiate binding was completely inhibited after the release of only 5 n*M* fatty acid per milligram membrane protein. Similarly, Lin and Simon (1978) showed the importance of long-chain fatty acids for opiate binding. They found that phospholipase A inhibited opiate binding; removal of the released fatty acids with albumin restored the binding.

Long-chain polyunsaturated fatty acids are also implicated in the action of ACTH on the adrenal cortex (Laychock *et al.*, 1978; Schrey and Rubin, 1979). $ACTH_{1-24}$ specifically stimulated the turnover of the pool of arachidonic acid that is bound to PI (Schrey and Rubin, 1979). This effect was dependent on extracellular Ca^{2+} and could be mimicked by the Ca^{2+} ionophore A23187. No effect of cAMP could be found and there seemed to be no relation with the PI response. This increased turnover of arachidonoyl-PI was mediated by a Ca^{2+}-dependent phospholipase A_2 (Rubin *et al.*, 1979) and the authors proposed that this accelerated turnover is an early and specific event in the action of ACTH.

It has been shown (Section III,E) that pituitary peptides can specifically interact with membrane lipids. Schoch *et al.* (1979) exposed $ACTH_{1\text{-}24}$ to one side of an artificial lipid bilayer and found that part of the molecule became exposed to the other (trans) side of this artificial membrane. It was presumed that the N-terminal region must be associated with the trans side mainly because the cluster of four positive charges at residues 15–18 would present a formidable barrier to translocation through the hydrophobic interior of the membrane. The peptide–lipid interaction was enhanced in the presence of negative phospholipids. Also Arnaud *et al.* (1980) showed that ACTH is able to interact with these hydrophobic membrane components.

IV. CONCLUDING REMARKS

The pituitary hormones and their fragments influence a variety of biochemical events in membranes (Section III). The question arises are these different molecular events related and do they reveal something about the physiological action of the pituitary hormones *in vivo?*

As a potent tool to bridge the gap between the behavioral action of the neuropeptides and their neurochemical effects, the structure–activity study was used. By the use of this pharmacological technique, it has been possible to discern different regions in the polypeptide structure of ACTH, MSH, and LPH that code for different CNS effects. For instance, both from the effects of ACTH fragments on the behavior (Section II,D) and from the biochemical studies it was concluded that the peptide $ACTH_{1\text{-}24}$ contains two affinity sites. Clearly, the *in vitro* effects paralleled those obtained *in vivo;* both sites need to be present simultaneously for effects on grooming behavior (*in vivo*), and adenylcyclase, B-50 phosphorylation, and TPI/PA formation (*in vitro*); the present of either of the sites seems sufficient for effects on avoidance behavior and counteraction of morphine-induced analgesia (*in vivo*), and DPI formation (*in vitro*) (Fig. 5). This correlation may indicate that a change in the neurochemical parameters underlies the effects on the behavior. Finally we will address ourselves to the possible physiological significance of the observed *in vitro* effects.

Among the molecular correlates of pituitary hormone action, two membrane-associated phenomena seem to play a crucial role: the metabolism of calcium and the presence of anionic lipids. The polyanionic lipids DPI and TPI are very potent chelators of Ca^{2+} and Mg^{2+}. By virtue of this aspect these lipids are involved in binding these ions to the membrane (Hawthorne and Kai, 1970; Michell, 1975, 1979), and in regulating the de- or hyperpolarization of the membrane (Torda, 1974). In addition, PI seems to be of crucial importance for both basal and hormone-sensitive adenylate cyclase (Michell, 1975, 1979; Sandermann, 1978) and for ATPase activity (DePont *et al.*, 1978). Furthermore, PI

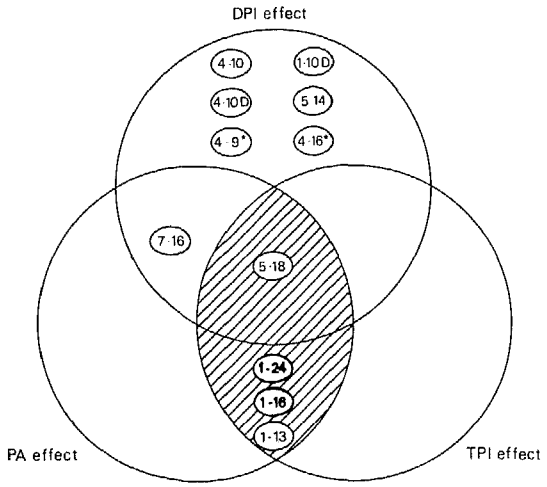

Fig. 5. Venn diagram showing the distribution of a number of ACTH fragments over three overlapping circles. Each circle represents a collection of ACTH fragments active on a particular aspect of polyphosphoinositide metabolism (stimulation of TPI formation; stimulation of DPI formation; inhibition of PA formation). The cross-hatched area represents the collection of ACTH fragments that is active on the phosphorylation of B-50 protein in synaptosomal plasma membranes (inhibition). The grey area represents the ACTH fragments that are active on adenylate cyclase activity in synaptosomal plasma membranes (inhibition). This figure suggests that different parts of the ACTH molecule can interact with different aspects of poly(PI) metabolism, B-50 protein phosphorylation, and adenylate cyclase activity. The sequences [Met(O)^4, D-Lys^8, Phe^9] $ACTH_{4-9}$ and [Met(O)^4, D-Lys^8, Phe^9, D-Lys^{11}] $ACTH_{4-16}$ are denoted as (4-9*) and (4-16*), respectively.

and its breakdown product 1,2-diacylglycerol activate a cytosolic protein kinase (Kishimoto *et al.*, 1980; Jolles *et al.*, 1980). The breakdown of PI results in the selective release of several membrane enzymes, indicating that PI is also involved in the anchoring of enzymes to the membrane (Arienti and Porcellatti, 1980; Fibean and Shukla, 1980; Kishimoto *et al.*, 1980; Michell, 1975, 1979). This anchoring may occur through the strong electrostatic interactions of the phosphatidylinositides with proteins via Ca^{2+} and Mg^{2+}. Anionic lipids (PI, TPI, and cerebroside sulfate) may also be an integral part of membrane receptors for several hormones such as thyrotropin, cholinerigic drugs, and opiates (Kohn, 1978; Abood *et al.*, 1978, Loh *et al.*, 1978). ACTH and βE seem able to interact with hydrophobic membrane constituents, especially negative lipids and direct effects of the neuropeptides on poly(PI) metabolism have been obtained (Farese *et al.*, 1979; Jolles, 1980; Jolles *et al.*, 1980, 1981a,b; Lo *et al.*, 1979; Shoch *et al.*, 1979).

Taken together, the presence of PI (and its derivative) determines the activity of membrane enzymes, the binding characteristics of membrane receptors, and

the binding and permeability of Ca^{2+}. As the poly(PI) are rapidly interconverted (Fig. 4) (the enzymes involved in their metabolism are among the fastest acting known; Hawthorne and Kai, 1970) these lipids play a key role in membrane functioning. Consequently, the dynamic properties of the membrane can be regulated by agents that alter the phosphorylation or breakdown of phosphatidylinositol.

In our view, the experimental evidence that has been provided in Sections II and III supports the notion that neuropeptides like ACTH and βE modulate brain function by virtue of their influence on poly(PI) metabolism. A change in protein phosphorylation may mediate this influence, with a change in cAMP production as a secondary consequence. The experimental support comes from (1) the similar structure–activity relationship on the *in vivo* and *in vitro* parameters, (2) the fact that both $ACTH_{1\text{-}24}$ and βE are partial agonist–antagonist for the opiate receptor, (3) the affinity of both peptides for hydrophobic membrane constituents, (4) the proposed lipid–protein character of the opiate receptor, (5) the effect of *in vivo* administered ACTH on phosphorylation of B-50 *in vitro*, (6) the causal relation between B-50 protein phosphorylation and poly(PI) metabolism, (7) the copurification of the ACTH-sensitive B-50/B-50 kinase and the lipid kinases, (8) the sensitivity of the partially purified enzyme complex for βE, (9) the regulation of the dynamic properties of the membrane by Ca^{2+} and anionic lipids, (10) the calcium-sensitive effects of ACTH on adenylate cyclase activity in broken cell preparations.

We propose the following working hypothesis for the action of ACTH-like neuropeptides: the peptide (e.g., $ACTH_{1\text{-}24}$) interacts with a mixed protein–lipid receptor. The protein part of the receptor is the regulatory subunit of a DPI kinase, and the lipid part may be a phosphoinositide, or possibly cerebroside sulfate. As a result of the peptide–receptor interaction, the autophosphorylation of its regulatory subunit (the B-50 protein) is inhibited, which results in activation of the DPI kinase (possibly by dissociation of the holoenzyme). TPI is formed and the breakdown of PI is inhibited, resulting in an inhibited Ca^{2+} influx, and inhibition of secondary Ca^{2+}-dependent processes (like cAMP production). The relationship with the inhibited PA formation is not totally clear at present, though it is probable that both processes are manifestations of an inhibited PI breakdown and inhibited Ca^{2+} influx. In the view of Michell (1975) certain extracellular agents (e.g., muscarinic cholinergic or α-adrenergic) act to enhance intracellular Ca^{2+} by the breakdown of PI to 1,2-daG. The consequent membrane depolarization and enhanced phosphorylation of 1,2-daG to PA reflects the breakdown of PI. The influx of Ca^{2+} leads to a rapid breakdown of the polyphosphoinsitides (DPI and TPI) at the cytoplasmic side of the membrane (Gritfin and Hawthorne, 1978; Abdel-Latif *et al.*, 1978). Consequently, the agonist-induced increase in PA formation and the decrease in TPI are a manifestation of the same phenomenon (see Soukup *et al.*, 1978; Jolles *et al.*, 1981c). Clearly, the effects of the neuropeptides are opposite to those

described for the classical neurotransmitters in that $ACTH_{1-24}$ and βE increase TPI and decrease PA. This could indicate that neuropeptides inhibit the Ca^{2+} influx (Guerrero-Munoz *et al.*, 1979), thereby hyperpolarizing the membrane (Torda, 1974) and acting to modulate the effects of the classical neurotransmitters.

It should be stated clearly that this is a working hypothesis; we have tried to fit the pieces of a puzzle together, but more research is needed to substantiate the notion that the neurochemical events are indeed related. For instance, it is not clear whether the modulatory action of the neuropeptides is located pre- or postsynaptically, as the present methods of tissue fractionation do not allow the proper separation of membranes from pre- and postsynaptic origin. Furthermore, though a correlation exists between *in vivo* and *in vitro* effects of the neuropeptides, a causal relation should be established, to determine whether the neurochemical events underlie the physiological action of the behaviorally active neuropeptides.

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